

APPROVED	O. G. FIG.
BY	CLASS
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le: Production of Human Parathyroid Hormone  
 From Microorganisms  
 Inventor(s): Kaare M. GAUTVIK, et al.  
 Appl. No.: 09/287,332  
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# **FIG. 1**

DNA SEQUENCE FOR HUMAN  
 PREPROPARATHYROID HORMONE

```

      10              30              50
ATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNATHTGYTTYTN

      70              90              110
ACNAARWSNGAYGGNAARWSNGTNAARAARMGNNWSNGTNWSNGARATHCARYTNATGCAY

      130             150             170
AAYYTNGGNAARCAYYTNAAYWSNATGGARMGNGTNGARTGGYTNGMNAARAARYTNCAR

      190             210             230
GAYGTNCAYAAAYTTYGTNGCNYTNGGNGCNCNYTNGCNCNM6NGAYGCNGGNNNCAR

      250             270             290
MGNCNM6NAARAARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGAR

      310             330
GCNGAYAARGCNGAYGTNAAYGTNYTNACNAARGCNAARWSNCARTTR
  
```

M = A OR C  
 R = A OR G  
 W = A OR T  
 S = C OR G  
 Y = C OR T  
 H = A OR C OR T  
 N = A OR G OR C OR T

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## FIG. 2

DNA SEQUENCE FOR HUMAN  
PREPROPARATHYROID HORMONE IN PLASMID pSSHPTH-10

```

      10              30              50
TATGATGATACCTGCAAAAGACATGGCTAAAGTTATGATTGTCATGTTGGCAATTTGTT

      70              90              110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCGTGGAGTGAATACAGCTTAT

      130             150             170
GCATAACCTGGGAAAACATCTGAACCTCGATGGAGAGAGTAGAATGGCTGCGTAAGAAGCT

      190             210             230
GCAGGATGTGCACAATTTTGTGCGCTTGGAGCTCCTCTAGCTCCAGAGATGCTGTTT

      250             270             290
CCAGAGGCCCCGAAAAAGGAAGACAATGTCTTGTTGAGAGCCATGAAAAAGTCTTGA

      310             330
GAGGCAGACAAAGCTGATGTGAATGTATTAAGCTAAATCCCAAGTGA

```

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### FIG. 3

PORTION OF DNA SEQUENCE OF THE PLASMID  
FOR INSERTION INTO E. COLI, CODING FOR HUMAN  
PREPROPARATHYROID HORMONE WITH FLANKING SEQUENCES.

10 30 50  
TATGATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNATHGTGTT

70 90 110  
YYTNACNAARWSNGAYGGNAARWSNGTNAARAARWSNGTNSNGARATHCARYTNAT

130 150 170  
GCAYAAYTTNGGNAARCAYYTNAAYWSNATGGARWGNGTNGARTGGYTNGNAARAARYT

190 210 230  
NCARGAYGTNCAYAAYTTYGTNGCNYTNGGNGCNCNYTNGCNCNMGNAYGCNGGNWS

250 270 290  
NCARWGNCNMGNARAARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGG

310 330 350  
NGARGCNGAYAARGCNGAYGTNAAYGTNYTNACNAARGCNAARWSNCARTPRAAATGAAA

370 390 410  
ACAGATATTGTGAGATTCTGCTCTAGACAAGTGTAGGGCAACAATACATGCTGCTAATTC

430  
AAAGCTCTATTA

M = A OR C  
R = A OR G  
W = A OR T  
S = C OR T  
Y = C OR T  
H = A OR C OR T  
N = A OR G OR C OR T

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# **FIG. 4**

DNA SEQUENCE FOR HUMAN PREPROPARATHYROID  
 HORMONE IN PLASMID pSSHPTH-10 WITH FLANKING SEQUENCES

```

      10              30              50
TATGATGATACCTGCAAAAGACATG6CTAAAGTTATGATTGTCATGTTG6CAATTGTTT

      70              90              110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAATACAGCTTAT

      130             150             170
GCATAACCTG6GAAAACATCTGAACTCGATGGAGAGAGTAGAATG6CTGCGTAAGAAGCT

      190             210             230
GCAGGATGTGCACAATTTTGTGCCCCTGGAGCTCCTCTAGCTCCAGAGATGCTG6TTC

      250             270             290
CCAGAGGCCCGAAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAGTCTTGG

      310             330             350
AGAGGCAGACAAAGCTGATGTGAATGTATTAAGTAAAGCTAAATCCAGTGAAAATGAAA

      370             390             410
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTC

      430
AAAGCTCTATTA
  
```

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# **FIG. 5**

DNA SEQUENCE CODING FOR PREPROPARATHYROID HORMONE  
 IN pSSHPTH-10 WITH FLANKING SEQUENCES, SHOWING  
 THE CORRESPONDING AMINO ACID SEQUENCE OF  
 PREPROPARATHYROID HORMONE

10	30	50
TATGATGATACCTGCAAAAGACATGGCTAAAGTTATGATTGTCATGTTGGCAATTTGTTT		
MetIleProAlaLysAspMetAlaLysValMetIleValMetLeuAlaIleCysPh		
70	90	110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAATACAGCTTAT		
eLeuThrLysSerAspGlyLysSerValLysLysArgSerValSerGluIleGlnLeuMe		
130	150	170
GCATAACCTGGGAAAACATCTGAACTCGATGGAGAGAGTAGAATGGCTGCGTAAGAAGCT		
tHisAsnLeuGlyLysHisLeuAsnSerMetGluArgValGluTrpLeuArgLysLysLe		
190	210	230
GCAGGATGTGCACAATTTTGTGGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTC		
uGlnAspValHisAsnPheValAlaLeuGlyAlaProLeuAlaProArgAspAlaGlySe		
250	270	290
CCAGAGGCCCGGAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAGTCTTGG		
rGlnArgProArgLysLysGluAspAsnValLeuValGluSerHisGluLysSerLeuGln		
310	330	350
AGAGGCAGACAAAAGCTGATGTGAATGTATTAAGTAAAGCTAAATCCAGTGAAAAATGAAA		
yGluAlaAspLysAlaAspValAsnValLeuThrLysAlaLysSerGlnEnd		
370	390	410
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTC		
430		
AAAGCTCTATTA		

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## FIG. 6

NUCLEOTIDE SEQUENCE OF THE MF 1-HPTH FISION-GENE FROM pS LX5-HPTH1.  
 NUCLEOTIDE NOS. 1-173 MAKEUP THE MH 1 PROMOTER REGION AND 5' NONCODING SEQUENCE. 174-440 IS THE MF 1 N-TERMINAL CODING SEQUENCE. 441-695 IS THE HPTH SEQUENCE OBTAINED FROM pSSHPTH-10. 696-726 IS AN HPTH 3' NONCODING SEQUENCE FROM pSSHPTH-10. 727-732 IS FROM pUC19. 733-874 IS MF 1 3' NONCODING SEQUENCE AND TRANSCRIPTIONAL TERMINATION SIGNAL.

```

      10              30              50
AGTGAAGAAAACAAAAAGCAACAACAGGTTTTGGATAAGTACATATATAAGAGGGCCT
      70              90              110
TTTGTTC CATCAAAAATGTTACTGTTCTTACGATTCATTTACGATTCAAGAATAGTTCA
     130             150             170
AACAGAAGATTACAACTATCAATTTATACACAATATAAACGACCAAAAGAATGAGAT
     190             210             230
TTCCTTCAATTTTTACTGCAGTTTTATTGCGAGCATCCTCCGCATTAGCTGCTCCAGTCA
     250             270             290
ACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTCAG
     310             330             350
ATTTAGAAGGGGATTTGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGT
     370             390             410
TATTGTTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTTTGG
     430             450             470
ATAAAAGAGAGGCTGAAGCTTCTGTGAGTGAAATACAGCTTATGCATAACCTGGGAAAAC
     490             510             530
ATCTGAACCTCGATGGAGAGAGTAGAATGGCTGCGTAAGAAGCTGCAGGATGTGCACAATT
     550             570             590
TTGTTGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTCAGAGGGCCCCGAAAAA
     610             630             650
AGGAAGACAATGTCTTGTTGAGAGCCATGAAAAAAGTCTTGAGAGGCAGACAAAGCTG
     670             690             710
ATGTGAATGTATTAATAAGCTAAATCCAGTGAAAATGAAAACAGATATTGTCAGAGT
     730             750             770
TCTGCTCTAGAGTCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATAC
     790             810             830
TTTTCAATTTCTCCGTAAACAACCTGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTT
     850             870
CGTTACCAACTTTACACATACTTTATATAGCTAT

```

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## FIG. 7

PARTIAL DNA SEQUENCE FOR THE PLASMID FOR INSERTION INTO YEAST  
IN WHICH: NUCLEOTIDE NOS. 1-173 MAKEUP THE MF 1 PROMOTER  
REGION AND 5' NONCODING SEQUENCE. 174-440 IS THE MF 1 N-TERMINAL  
CODING SEQUENCE. 441-695 IS AN HPTH SEQUENCE. 696-726 IS AN  
HPTH 3' NONCODING SEQUENCE FROM pSSHPTH-10. 727-732 IF FROM  
PUC19. 733-874 IS MF 1 3' NONCODING SEQUENCE AND TRANSCRIPTIONAL  
TERMINATION SIGNAL.

```

10          30
AGTGAAGAAAACCAAAAAGCAACAACAGGTTTTGGATAAGTACATATATAAGAGGGCCT
70          90          110
TTTGTTCATCAAAAATGTTACTGTTCTTACGATTCATTTACGATTCAAGAATAGTTCA
130         150         170
AACAGAAGATTACAACTATCAATTTATACACAATATAAACGACCAAAAGAATGAGAT
190         210         230
TTCCTTCAATTTTACTGCAGTTTTATTTCGAGCATCCTCCGATTAGCTGCTCCAGTCA
250         270         290
ACACTACAACAGAAGATGAAACGGCACAAATTCGGCTGAAGCTGTCATCGGTTACTCAG
310         330         350
ATTTAGAAGGGGATTTGATGTTGCTGTTTTGCCATTTCCAACAGCACAAATAACGGGT
370         390         410
TATTGTTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTTTGG
430         450         470
ATAAAGAGAGGCTGAAGCTWSNGTWSNGARATHCARYTNATGCAYAAYYTNGGNAARC
490         510         530
AAYTNAAYWSNATGGARMNGTNGARTGGYTNGMNAARAARYTNCARGAYGTNCAYAAYT
550         570         590
TYGTNGCNYTNGGNGCNCNYTNGCNCNMNGAYGCNGGWSNCARMGNCNMGNAARA
610         630         650
ARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGARGCNGAYAARGCNG
670         690         710
AYGTNAAYGTNYTNACNAARWSNCARTTAAATGAAAACAGATATTGTCAGAGT
730         750         770
TCTGCTCTAGAGTCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATAC
790         810         830
TTTTCATTTCTCCGTAAACAACCTGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTT
850         870
CGTTACCAACTTTACACATACTTTATATAGCTAT, WHEREIN

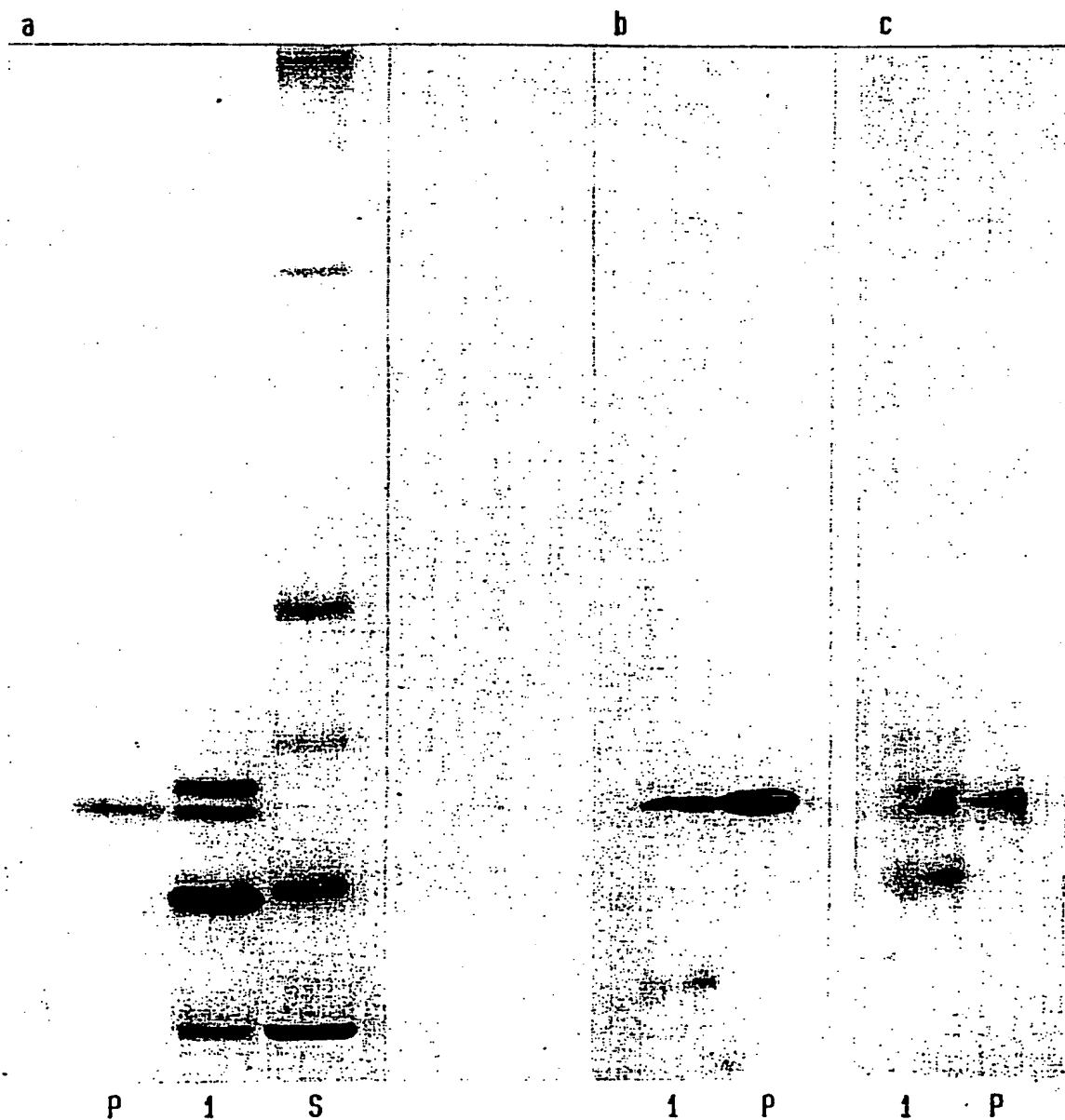
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M = A OR C  
R = A OR G  
W = A OR T  
S = C OR G  
Y = C OR T  
H = A OR C OR T  
N = A OR G OR C OR T

APPROVED	FIG.	
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**FIG. 8**



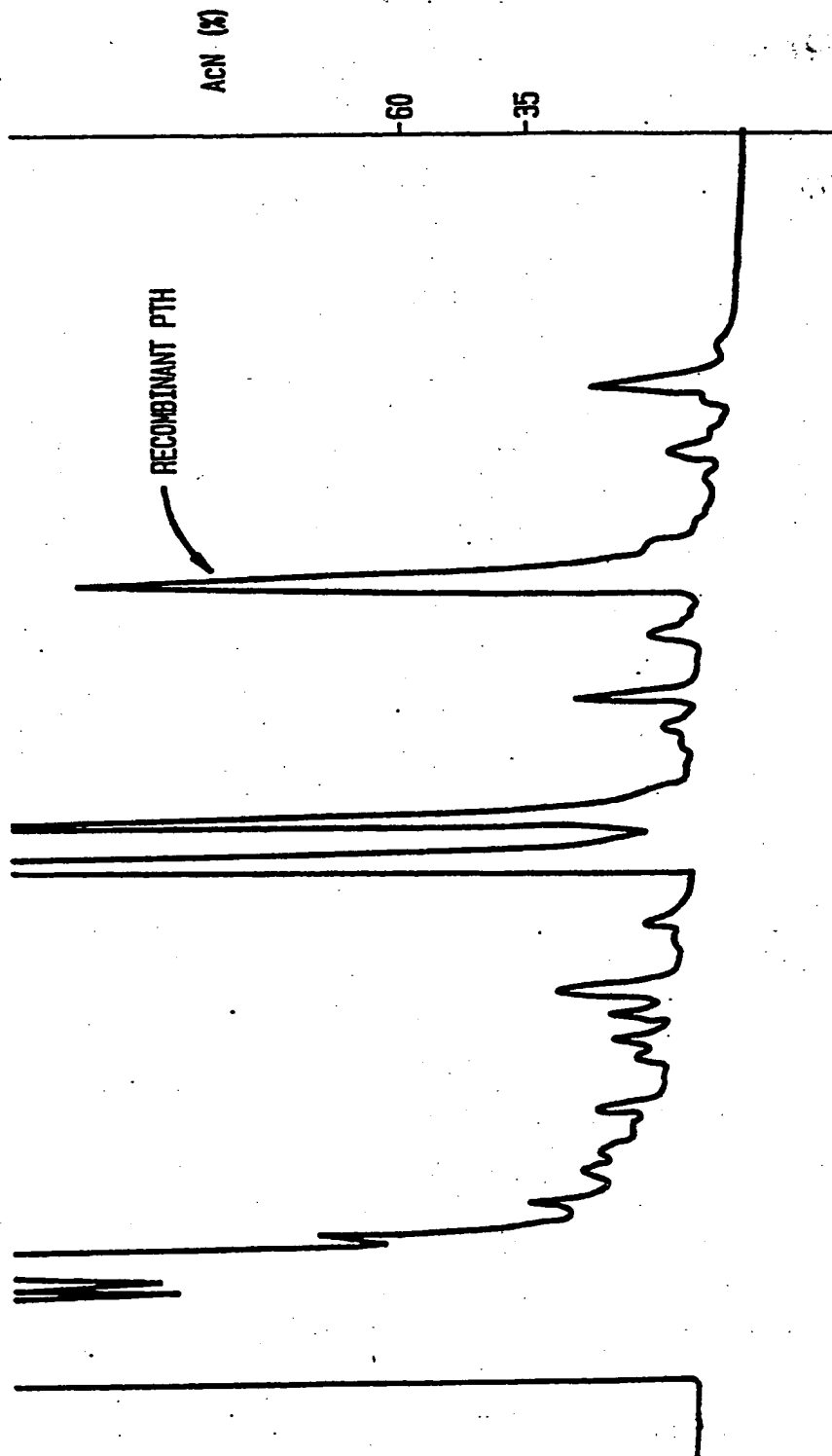


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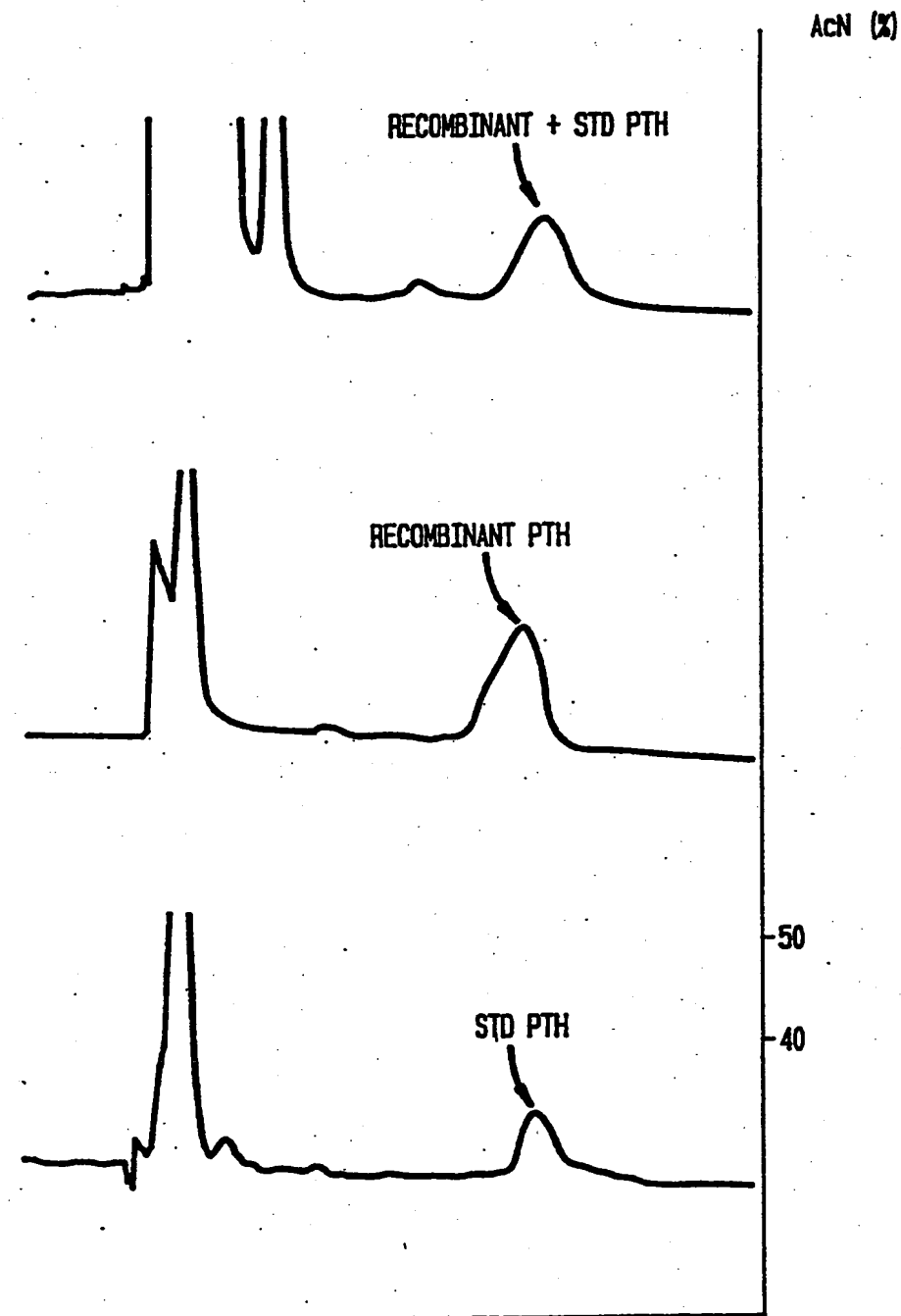
FIG. 9A



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**FIG. 9B**



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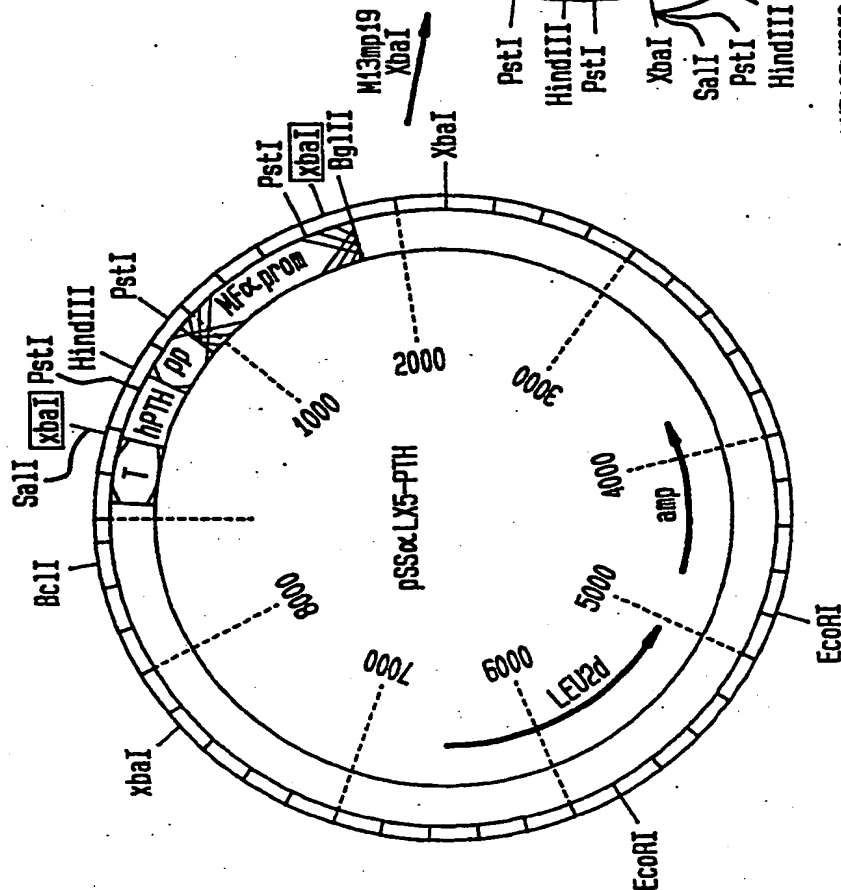
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Inventor(s): Kaare M. GAUTVIK, et al.

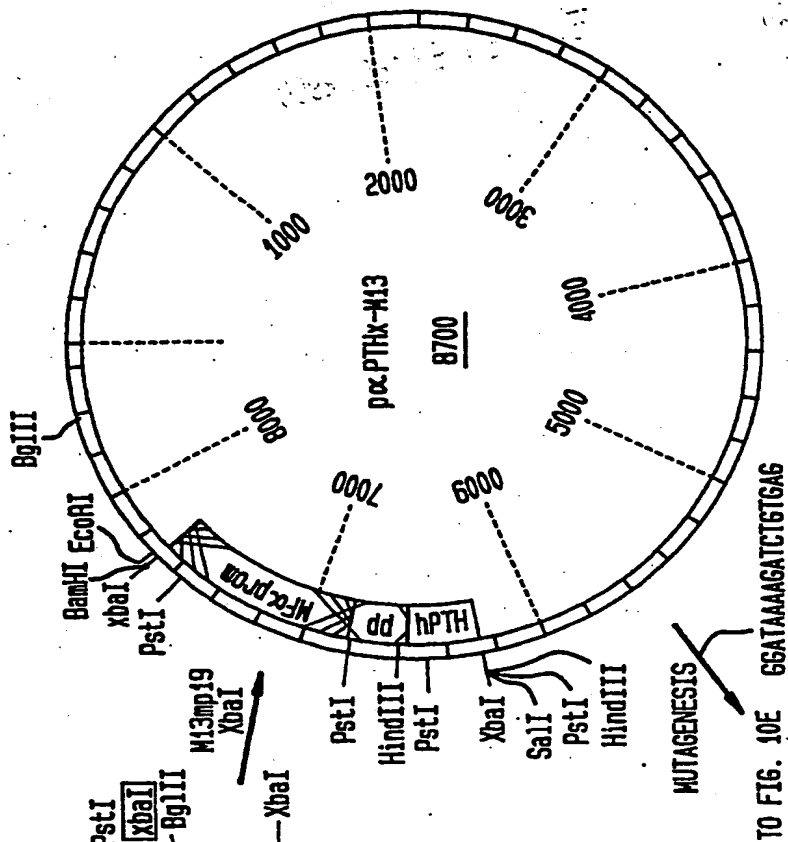
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**FIG. 10A**



**FIG. 10B**



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# Title: Production of Human Parathyroid hormone From Microorganisms

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FIG. 10C

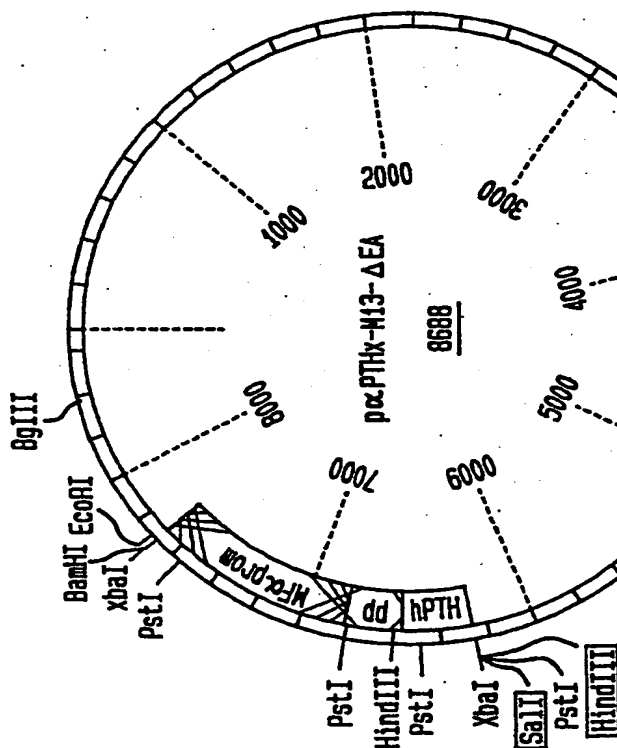
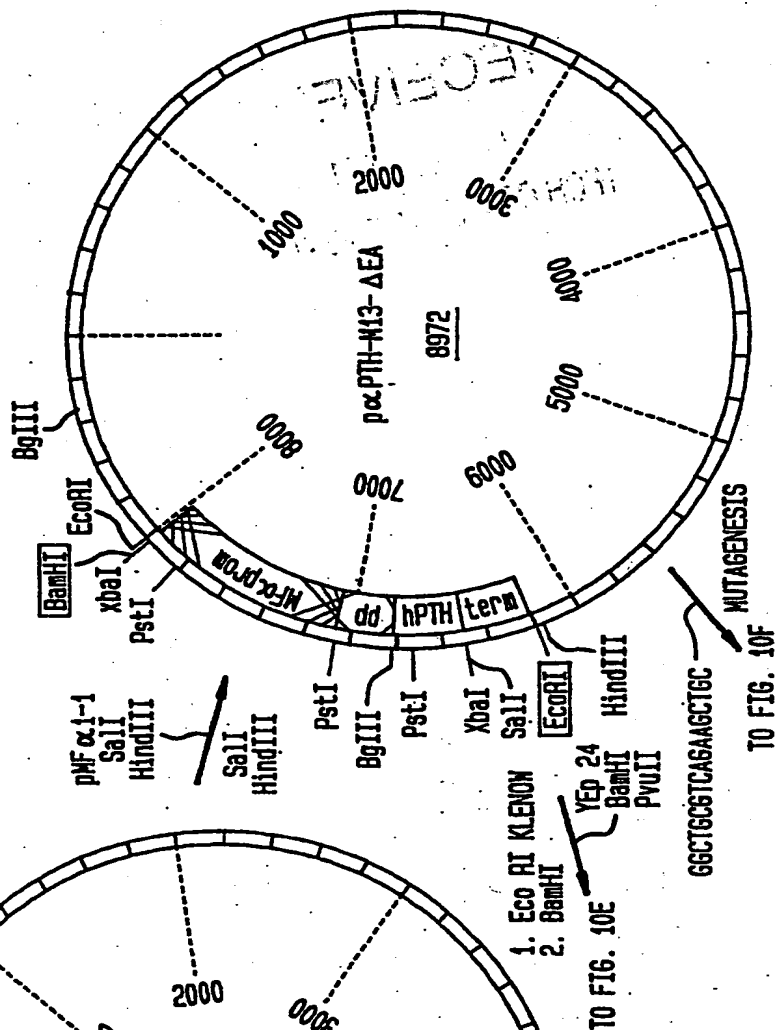


FIG. 10D



TO FIG. 10E

1. Eco RI KLENOW  
2. BamHI

YEp 24

BamHI

PvuII

GGCTGGCTCAGAGCTGC

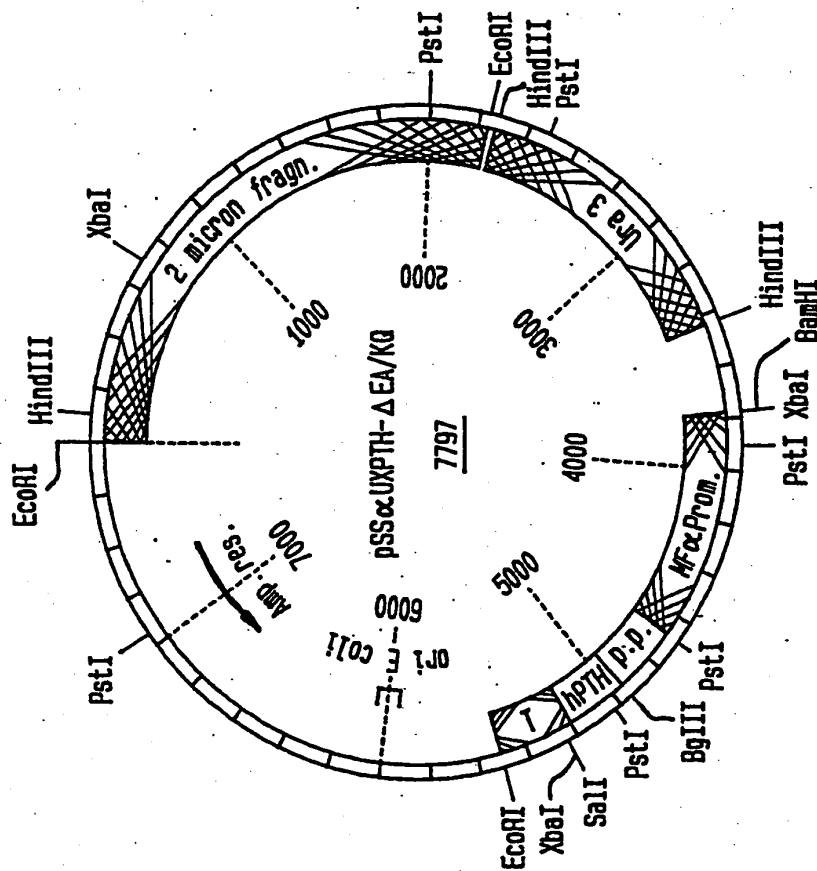
TO FIG. 10F



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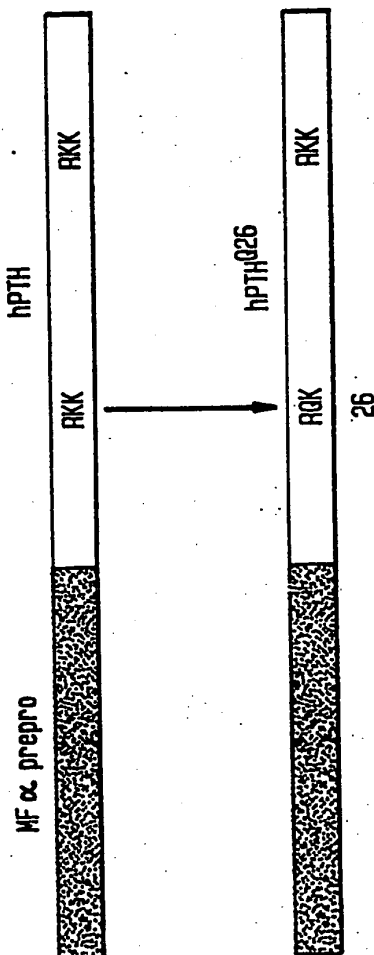
FIG. 10G



APPROVED	O. G. FIG.	
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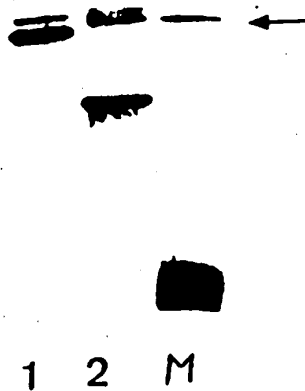
**FIG. 11**  
IN vitro MUTAGENESIS OF hPTH



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**FIG. 12**



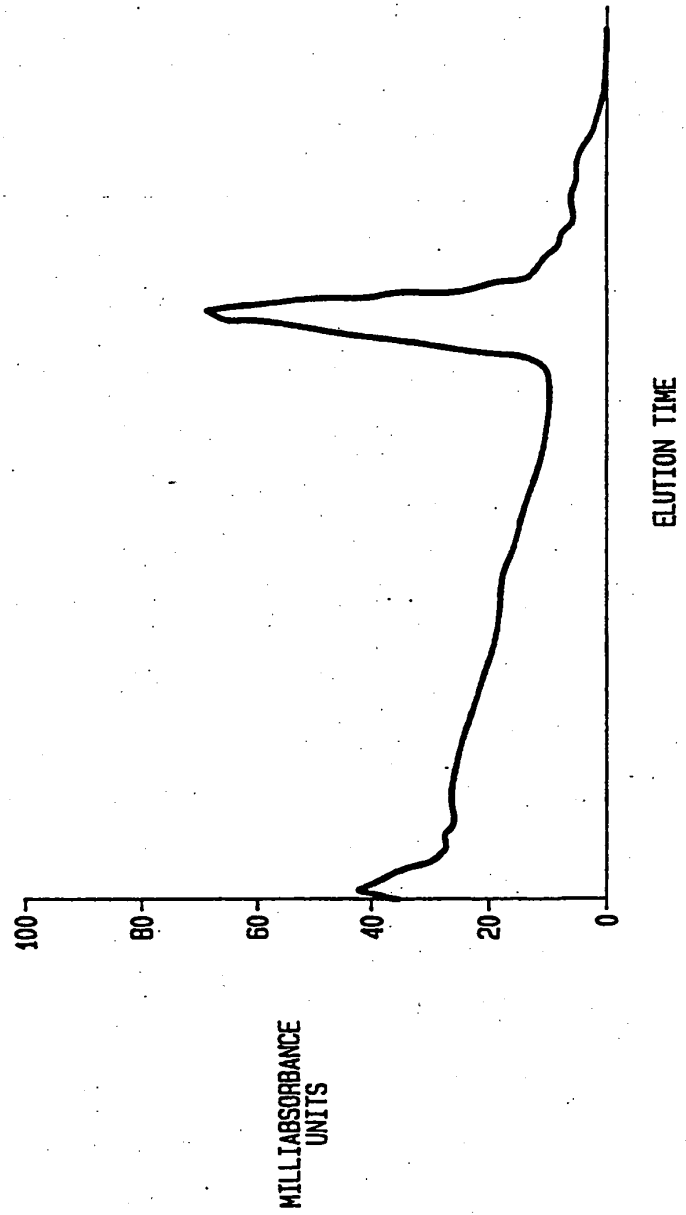


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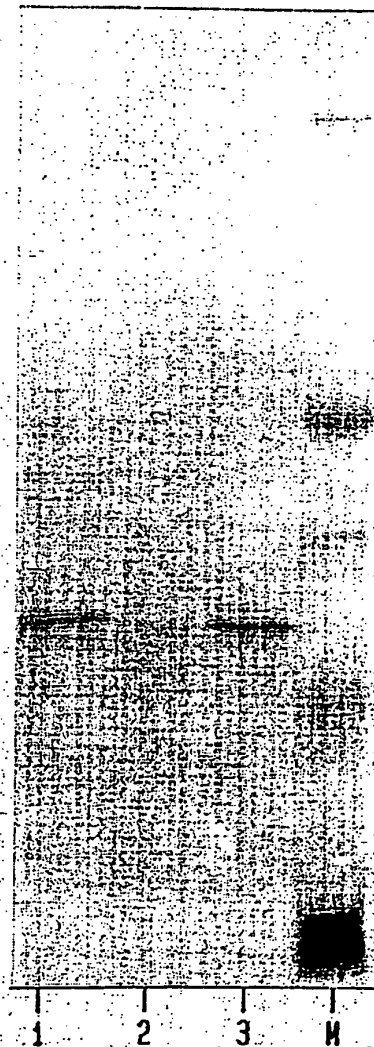
**FIG. 13A**  
 HPLC CHROMATOGRAM OF hPTH (Q26)



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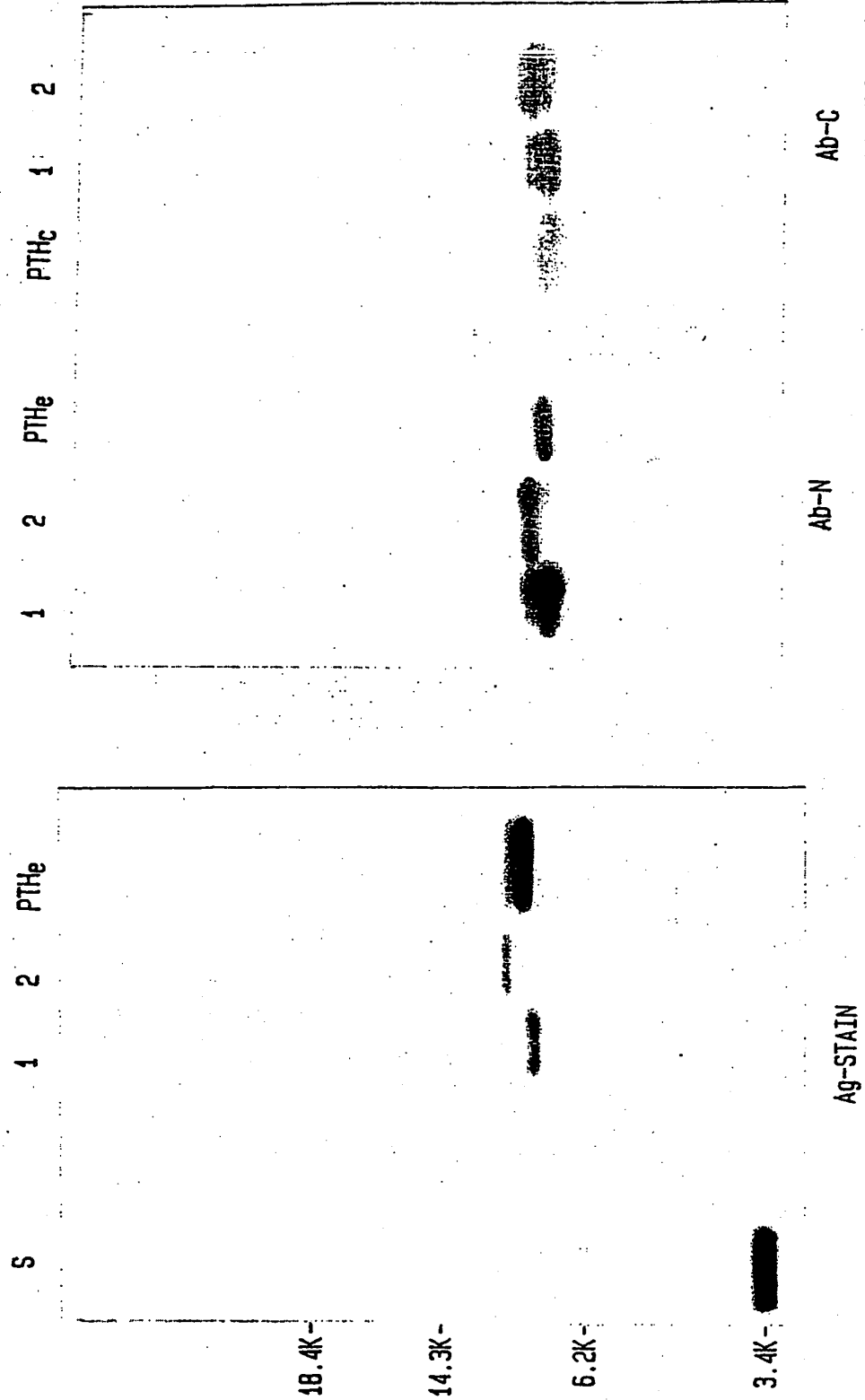
**FIG. 13B**



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 From Microorganisms  
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FIG. 14



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FIG. 15

